IN THE CLAIMS:

1. (Original) A process for extracting native or recombinantly-expressed, gram-negative inner membrane proteins from bacteria or bacterial host cells containing a recombinant vector by differential detergent tangential flow diafiltration, which comprises:

(a) lysing bacteria or bacterial host cells containing a recombinant vector in a fermentation broth;

(b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;

(c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization; and

(d) collecting the inner membrane proteins removed in (c).

2. (Previously presented) The process of Claim 1 wherein: the lysis of (a) occurs in a microfluidizer; in (b), the buffer is selected from the group consisting of Hepes, 3-(N-morpholino)propane sulfonic acid (MOPS), Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate; and in (c), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside, and

the divalent cation is selected from the group consisting of magnesium and calcium (Mg^{+2} and Ca^{+2}).

3. (Previously presented) The process of Claim 2 wherein in (b), the buffer is Hepes and the chelating agent is EDTA; and in (c), the buffer is Hepes, the detergent is alpha-[4-(1,1,3,3,-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl), and the divalent cation is Mg^{+2}.

4. (Original) A process for extracting native or recombinantly-expressed, gram-negative outer membrane proteins from bacteria or bacterial host cells containing a recombinant vector by differential detergent tangential flow diafiltration, which comprises:

(a) lysing bacteria or bacterial host cells containing a recombinant vector in a fermentation broth;

(b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;

(c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization;

(d) diafiltering the lysate from (c) with the buffer from (c), and using a divalent cation from (c) in the absence of detergent, in order to reduce the concentration of the detergent from (c);

(e) diafiltering the lysate from (d) with a buffer which is not retained by the diafiltration membrane, a chelating agent and a detergent to solubilize and remove the outer membrane proteins; and

(f) collecting the outer membrane proteins removed in (e).

5. (Previously presented) The process of Claim 4 wherein: the lysis of (a) occurs in a microfluidizer; in (b), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate; in (c), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside, and the divalent cation is selected from the group consisting of Mg^{+2} and Ca^{+2}; in (d), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, and the divalent cation is selected from the group consisting of Mg^{+2} and Ca^{+2}; and in (e), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, and the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside.

6. (Currently amended) The process of Claim ~~3~~ 4 wherein in (b), the buffer is Hepes and the chelating agent is EDTA; in (c), the buffer is Hepes, the detergent is alpha-[4-(1,1,3,3,-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl), and the divalent cation is Mg^{+2}; in (d), the buffer is Hepes and the divalent cation is Mg^{+2}; and in (e), the buffer is

Tris(hydroxymethyl)aminomethane, the chelating agent is EDTA, and the detergent is *n*-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate.

7. (Original) The process of Claim 4, which further comprises:

(g) diafiltering the lysate from (e) with reagents of (e), with the exception of the detergent, in order to reduce the concentration of the detergent;

(h) diafiltering the lysate from (g) with reagents of (e); and

(i) collecting the outer membrane proteins removed in (h).

8. (Original) A process for extracting lipidated recombinant outer membrane protein P4 (rP4) of *Haemophilus influenzae* from bacterial host cells by differential detergent tangential flow diafiltration, which comprises:

(a) lysing bacterial host cells in a fermentation broth;

(b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;

(c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization;

(d) diafiltering the lysate from (c) with the buffer from (c), and using a divalent cation from (c) in the absence of detergent, in order to reduce the concentration of the detergent from (c);

(e) diafiltering the lysate from (d) with a buffer which is not retained by the diafiltration membrane, a chelating agent and a detergent to solubilize the outer membrane proteins;

(f) diafiltering the lysate from (e) with a buffer which is not retained by the diafiltration membrane, a chelating agent and a detergent to extract and remove the lipidated rP4; and

(g) collecting the lipidated rP4 removed in (f).

9. (Previously presented) The process of Claim 8 wherein: the lysis of (a) occurs in a microfluidizer; in (b), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate; in (c), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside, and the divalent cation is selected from the group consisting of Mg^{+2} and Ca^{+2}; in (d), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, and the divalent cation is selected from the group consisting of Mg^{+2} and Ca^{+2}; in (e), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, and the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside; and in (f), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, and the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent compound, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside.

10. (Previously presented) The process of Claim 9 wherein in (b), the buffer is Hepes and the chelating agent is EDTA; in (c), the buffer is Hepes, the detergent is alpha-[4-(1,1,3,3,-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl), and the divalent cation is Mg^{+2}; in (d), the buffer is Hepes and the divalent cation is Mg^{+2}; in (e), the buffer is Tris(hydroxymethyl)aminomethane, the chelating agent is EDTA, and the detergent is *n*-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; and in (f), the buffer is Tris(hydroxymethyl)aminomethane, the chelating agent is EDTA, and the detergent is *n*-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate.

11. (Original) The process of Claim 8, which further comprises:

(h) diafiltering the lysate from (f) with reagents of (f), with the exception of the detergent, in order to reduce the concentration of the detergent;

(i) diafiltering the lysate from (h) with reagents of (f) to extract and remove the lipidated rP4; and

(j) collecting the lipidated rP4 removed in (i).

12. (Original) The process of Claim 11, which further comprises:

(k) diafiltering the lysate from (j) with reagents of (f), with the exception of the detergent, in order to reduce the concentration of the detergent;

(l) diafiltering the lysate from (k) with reagents of (f) to extract and remove the lipidated rP4; and

(m) collecting the lipidated rP4 removed in (l).

13. (Original) A process for extracting lipidated recombinant outer membrane protein P6 (rP6) of *Haemophilus influenzae* from bacterial host cells by differential detergent tangential flow diafiltration, which comprises:

(a) lysing bacterial host cells in a fermentation broth;

(b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;

(c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization;

(d) diafiltering the lysate from (c) with a buffer which is not retained by the diafiltration membrane, a chelating agent to sequester divalent cation and to prevent proteolysis, and a detergent to solubilize and remove the outer membrane proteins other than lipidated rP6;

(e) diafiltering the lysate from (d) with a buffer which is not retained by the diafiltration membrane, a chelating agent to prevent proteolysis, a detergent to remove additional outer membrane proteins, and a salt to disrupt the membrane/outer membrane protein complex;

(f) diafiltering the lysate from (e) with reagents of (e), with the exception of the detergent and the salt, in order to reduce the concentration of the detergent;

(g) diafiltering the lysate from (f) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes

and removes additional proteins bound to the cellular outer membrane, and using a chelating agent to prevent proteolysis;

(h) diafiltering the lysate from (g) with the buffer from (g) and the chelating agent of (g) to reduce the concentration of the detergent from (g);

(i) diafiltering the lysate from (h) with a phosphate compound and a detergent to solubilize and remove additional proteins bound to the cellular outer membrane;

(j) diafiltering the lysate from (i) with a phosphate compound to reduce the concentration of the detergent from (i);

(k) heating the lysate from (j) to remove lipidated rP6 from the membrane while diafiltering that lysate with a phosphate compound and a detergent to solubilize, extract and remove the lipidated rP6; and

(l) collecting the lipidated rP6 removed in (k).

14. (Previously presented) The process of Claim 13 wherein: the lysis of (a) occurs in a microfluidizer; in (b), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, and the divalent cation is selected from the group consisting of Mg^{+2} and Ca^{+2}; in (c), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside, and the divalent cation is selected from the group consisting of Mg^{+2} and Ca^{+2}; in (d), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, and the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl,

a glucoside compound, a cholate compound and dodecyl-maltoside; in (e), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, the salt is a sodium salt, and the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside; in (f), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate; in (g), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate, and the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside; in (h), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane, sodium phosphate and sodium borate; in (i), the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside; and in (k), the detergent is selected from the group consisting of a zwitterionic detergent, a non-ionic detergent, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside.

15. (Previously presented) The process of Claim 14 wherein in (b), the buffer is Hepes and the chelating agent is EDTA; in (c), the buffer is Hepes, the detergent is alpha-[4-(1,1,3,3,-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl), and the divalent cation is Mg^{+2}; in (d), the buffer is Hepes, the chelating agent is EDTA, and the detergent is *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; in (e), the buffer is Hepes, the chelating agent is EDTA, the salt is sodium chloride, and the detergent is *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; in (f), the buffer is Tris(hydroxymethyl)aminomethane and the chelating agent is EDTA; in (g), the buffer is Tris(hydroxymethyl)aminomethane, the

detergent is sarcosyl, and the chelating agent is EDTA; in (h), the buffer is Tris(hydroxymethyl)aminomethane and the chelating agent is EDTA; in (i), the detergent is *n*-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, and the phosphate is sodium phosphate; in (j), the phosphate is sodium phosphate; and in (k), the detergent is *n*-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate.

16. (Original) The process of Claim 13 wherein prior to (k), the lysate from (j) is concentrated.